A CONTRIBUTION TO THE EXPERIMENTAL PATHOLOGY OF CATARRHAL JAUNDICE

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The discovery by Bayliss and Starling[1] that the mechanism of pancreatic secretion was of the nature of a chemical reflex has opened up many important lines of research both in physiology and in experimental pathology. Stated in its most simple form, this mechanism consists in the formation in the upper part of the small intestines of a substance called Secretin, which, entering the blood stream and thereby coming eventually into contact with the cells of the pancreas, excites this organ to activity and produces a copious flow of juice. Secretin is only formed when acid is present in the contents of the duodenum and jejunum : the acid is supposed to act upon a ' mother substance ' prosecretin in the epithelial cells, and to produce therefrom this secretin. As long as acid is in contact with the mucous membrane in this tract of bowel, so long will secretin be formed ; eventually the alkaline pancreatic flow provoked by the secretin will neutralise the duodenal contents, and thereby further formation of secretin will cease. Later research has demonstrated that secretin is also a stimulant to the liver and intestinal glands.[2]

The acid, therefore, in the stomach not only acts as a necessary factor in peptic digestion, and also a trap for bacteria, but in a lower portion of the alimentary canal it is necessary for the proper stimulation of liver, pancreas, and intestinal glands.

Some important applications of this discovery to pathology have already been given by Starling,[3] but hitherto no attention has been

1. Bayliss and Starling, *Journal of Physiology*, Vol. XXVIII, 325, 1902.
2. Starling, Croonian Lectures, 1905.
3. Starling, *Trans. Pathol. Soc. London*, 1903.

given to its application to the experimental pathology of catarrhal jaundice. In catarrhal jaundice the most evident and obtrusive signs and symptoms are those due to stasis and re-absorption of bile. It is singular how little attention is paid to the result of obstruction of the pancreatic duct which occurs in this form of icterus, though the pancreatic juice contains three of the most powerful digestive enzymes, and is also concerned largely with the neutralization of the chyme by means of the alkaline carbonate which it contains.

In health the stomach contents are acid, but in the duodenum there is, as we have seen, an automatic mechanism, namely, secretin formation, which ensures neutrality. Here peptic digestion must obviously come to a standstill, as the acid reaction essential for its activity is no longer maintained. But secretin has not only summoned alkali for neutralization purposes, it has brought also in the pancreatic juice at least four enzymes which will proceed to effect hydrolysis and digestion of the neutralized duodenal chyme.

Suppose now (what actually happens in catarrhal jaundice) that both bile and pancreatic juice are prevented from entering the duodenum. If the stomach still secretes hydrochloric acid, then this acid will pass into tracts of bowel which have hitherto been accustomed to enclose neutral or only faintly acid or alkaline contents. This cannot fail to act in a disturbing manner.

If prosecretin continues to be present in the duodenum and jejunum, then the continued acidity of their contents will produce vigorous formation of secretin, which, if the obstructed organs still retain their sensitiveness to this stimulus, will continue to urge both pancreas and liver to greater activity although no direct outlet for the secretions is available. This increased formation of secretin will, however, have this important advantage, that the flow of succus entericus will be greatly increased, thereby neutralizing the acid jejunal contents and bringing large quantities of the proper ferments of the intestines to bear upon the merely peptonised chyme.

If, however, no acid is produced in the stomach, then no secretin will be formed, and the liver and pancreas will not be stimulated to useless efforts, and the contents of the duodenum and jejunum will

not possess an irritating acidity. Against these advantages there must be noted that peptic digestion will be abolished, that bacteria will have ready entrance, and that the proper stimulus to the intestinal glands will be absent.

The questions which such theoretical considerations raise may be grouped as follows :—

(1) If pancreatic duct or pancreatic and bile duct together be obstructed, is acid still found in the stomach ?

(2) If so, does prosecretin continue to be present in the mucous membrane of duodenum and jejunum ?

(3) Do the pancreas and liver continue to be responsive to secretin when egress for their secretion is denied ?

(4) Does acid chyme under such circumstances extend for any distance down the small intestine ?

Chemical Methods

Investigation of the stomach contents was confined solely to the relative amounts of hydrochloric acid secreted. No attempt was made to differentiate between free and ' combined ' hydrochloric. The term ' combined hydrochloric ' seems to cover not only the acid held in loose combination (adsorption or reversible molecular combination), which has still acidic properties—that is, has a certain hydrogen ion concentration—but also the more definite compound obtained by the proteid becoming basic and uniting with the acid to form a stable chloride. As I have been unable to discover any data giving the hydrogen concentration of these compounds, nor of the hydrogen ion concentrations, which are denoted by the end-points of the indicators employed, I have decided not to employ titrations such as those with alizarin and dimethyl-amido-azo-benzol, as they cannot but be empiric.

I have also noted in a dog on which oesophagostomy had been performed, that the animal remained in perfect health (and even laid on flesh) though no free hydrochloric acid was detectable by the Gunzberg reaction in the stomach after a meal rich in proteids. Here practically all the acid was combined with proteids. This fact

supports the contention of many physiologists that acid over and above what is necessary to saturate the proteids present is not an essential factor in gastric digestion. The method employed for estimating the hydrochloric acid was a modification of that of Lüttke, and had better be described in detail :—

A meal of steamed dog biscuit was given to the dog in the morning, and one and a half hours afterwards a sample of the stomach contents was withdrawn and filtered. Two equal portions, 'A' and 'B,' of the filtrate, measured by pipette, were placed each in a porcelain crucible. To 'B' slight excess of chloride-free sodium carbonate was added. Both were placed on a water bath, allowed to evaporate to dryness, and then calcined over a weak bunsen flame until all burning had ceased. The residue in each case was repeatedly extracted with distilled water. In each extract the chlorides present were estimated by the Volhardt method. That is to say, nitric acid in a few drops of iron-alum solution were added to each, and then a measured excess of $\frac{N}{10}$ silver nitrate.

The total volume of each was diluted with distilled water to the same volume, and one expressible in round numbers, then filtered, and half the filtrate taken and titrated with $\frac{N}{10}$ ammonium sulphocyanide until a red colour appeared.

The difference in each case between the amount of $AgNO_3$ and double the amount of AmCNS gives the amount of chlorides present in each calcined residue. The difference finally between the chlorides of each residue represents the amount of hydrochloric acid expressed in c.c. of decinormal strength present in the portion of filtered juice 'A' or 'B.' From this the normality of hydrochloric acid in the filtered juice can be calculated.

Criticism of the Method.—It will be seen from the above that this method of analysis aims at determining in a sample of the filtered contents of the stomach the amount of hydrochloric acid which has been added by the gastric glands.

As chlorides of the physiological metals are volatile at temperatures easily reached in calcination wrong results will be given if volatilisation occurs to a greater or less extent in 'A' than in 'B.' This can be obviated by using a low bunsen flame and taking care not to overheat during calcination.

A source of error which could not be easily obviated would arise if the food contained chlorides of organic bases, as these would decompose in heating, and be estimated as hydrochloric acid. In order to ascertain whether these were present as well as to test the method by a general control, an aqueous extract of the food was made, filtered, and analysed by the above method. The result of the analysis was to indicate that hydrochloric acid was present in a concentration equal to $\frac{N}{500}$ or 0·007%, a figure which may be taken to be within the limits of experimental error.

We may, therefore, legitimately assume that when an excess of chloride is found in 'B' (the juice to which sodium carbonate has been added) over 'A,' this excess represents total hydrochloric acid, free, combined, or both together.

Operative Procedures

Experiment I.—On the 15th February, 1905, a short-haired red terrier bitch was operated upon. After being morphinised and chloroformed, the anterior portion of the neck was shaved and sterilised, and an incision of 2 inches made in the mid-line.

In this operation I decided to perform oesophagostomy rather than oesophagotomy in order to be able to draw off the stomach contents[1]:—

(1) Because the saliva would not be so likely to bathe the wound, and so prevent rapid healing.

(2) Because the operation promised to be less severe on the animal.

(3) Because it appeared possible that the animal might learn to swallow its food in spite of the opening, and thus would be very much easier kept in a state of health, and approach more nearly to a normal condition.

The oesophagus was exposed and separated from its attachments just below the level of the thyroid cartilage, great care being exercised to avoid cutting the recurrent laryngeal nerve, as it lies on this part of the oesophagus.

The oesophagus being then drawn up into the wound, was opened by a longitudinal incision one inch in length down its centre, and a continuous suture of fine cat-gut was sewn right round the cut edges, so as to obliterate all raw surface.

The oesophagus was now sewn to the skin wound by cat-gut sutures passing through the sterno-mastoid muscles and out through the skin, so holding it in place.

A large opening was thus formed leading into the oesophagus, the suturing completely obliterating all raw surfaces, and the mucous membrane being closely approximated to the skin.

The wound was dressed with boracic acid and cotton wool and a gauze bandage, and it was remarkable with what rapidity healing took place, for in 24 hours the dog was lively and apparently well.

On February 22nd, several meals of bread having been given for a week—one meal each day being left in the stomach for one and a quarter hours—it was found that hydrochloric acid was present by qualitative tests. The dog kept in splendid condition, and except for the test meals of bread was fed on raw meat minced in a sausage machine.

After trying feeding by forcing the minced meat through a syringe for some time, it was found not only very troublesome, but the animal was scarcely getting enough.

Placing a pad of indiarubber over the oesophageal opening was then tried, and it was found that not only could the dog swallow minced meat when taken by the mouth, but it could eat small pieces equally well and lap water. Indeed, it was ultimately proved that the animal got so accustomed to the oesophageal opening that it could soon eat its meals as comfortably in the new condition as before operation. This materially assisted the course of the experiments, and made it much easier to keep the dogs in good health.

1. The operation of oesophagotomy described by Pawlow (*Ergebnisse der Physiologie* I, Abt. i, 246, 1902) had previously been performed on another dog, but the animal lost flesh and the neck wound did not heal well.

On 23rd February, after a test meal of bread, 20 c.c. of filtered gastric juice was quantitatively analysed for hydrochloric acid.

The amount of this acid stated in terms of normality was $\frac{N}{15.6}$.

On 25th February, 50 c.c. showed a concentration of hydrochloric acid equal to $\frac{N}{27.1}$.

On 27th February, 40 c.c. showed a concentration of hydrochloric acid equal to $\frac{N}{30.3}$.

From this time on till the 22nd May, the dog kept in perfect condition and was used at times for demonstration in class.

On the 22nd May, after being morphinised and anaesthetised by chloroform and ether mixture the abdomen was shaved, cleansed by scrubbing with ethereal soap and hot water, then with turpentine, and the skin made aseptic with biniodide of mercury solution 1-500. An incision 3 inches in length was made in the mid-line between the ensiform cartilage and umbilicus. The stomach and intestines were found to be greatly distended, probably because a sufficiently long interval was not allowed to elapse between the last meal and commencement of operation. This distention rendered the operation very difficult.

The duodenum was lifted and the pancreas exposed. The vessels near the duct were ligatured with silk and cut, and the duct dissected out, tied in two places with silk, and cut across between the ligatures.[1]

The abdominal wound was then closed in layers, as follows :—(*a*) The peritoneum was closed by a continuous cat-gut suture. (*b*) Two silk-worm gut sutures were then introduced through skin, muscle, and aponeuroses on the one side and brought out through aponeuroses, muscle, and skin on the other, and left untied in the meantime. (*c*) Muscle and aponeuroses were brought into apposition by a continuous cat-gut suture. (*d*) The skin by a continuous cat-gut. (*e*) The silk-worm gut sutures were then tied.

The wound was dry dressed with boracic powder, a single strip of gauze, and collodion Dry gauze was placed over this and a wide flannelette bandage applied. The dog never rallied from the operation and died on the third day from shock.

Experiment II.—On the 19th August, oesophagostomy was performed on a white fox terrier bitch, the same methods being employed as have already been given in detail. The dog recovered rapidly, and the wound healed by first intention. This dog, when received into the laboratory and when operated upon was in rather poor condition, so that, before making experiments it was decided to feed freely with raw meat in order to allow the animal to put on flesh. This was accordingly done in the manner already mentioned, namely, by holding a pad over the opening in the throat and giving the food, which was readily swallowed by the mouth.

1. The small duct of the pancreas which enters the duodenum near the pylorus was not tied, as it is of such small calibre that its influence upon digestion may be disregarded, whilst its anatomical position renders it very difficult to reach.

On the 7th September a test meal of steamed dog biscuit was given at 10.5, and at 11.35 the contents of the stomach were syphoned off and filtered. There remained 50 c.c. of clear fluid as filtrate. This after analysis showed a concentration of hydrochloric acid equal to $\frac{N}{25}$.

On the 9th September 40 c.c. filtrate of gastric contents showed a concentration of hydrochloric acid equal to $\frac{N}{35{\cdot}7}$.

On the 12th September employing the same technique as before described, the abdomen was opened. The stomach and bowels were in this case empty, and the duodenum and pancreas were easily drawn up outside the wound, and the pancreatic duct, after being dissected out, was simply tied with silk so as to occlude its lumen, but was not cut. The abdomen was closed in layers as before, but fine silk was used for the peritoneum instead of cat-gut.

15th September—The dog was very lively, in good condition, and apparently none the worse for the operation. After the usual test meal of steamed biscuit 80 c.c., the filtrate showed a complete absence of hydrochloric acid.

18th September—70 c.c. filtrate showed a concentration of hydrochloric acid equal to $\frac{N}{75}$.

20th September—20 c.c. filtrate showed a concentration of hydrochloric acid equal to $\frac{N}{22}$.

24th September—70 c.c. filtrate showed a concentration of hydrochloric acid equal to $\frac{N}{22}$.

6th November—The dog's health had so far been maintained in apparently perfect condition, but the wound in the neck having cicatrised up was again opened.

9th November—18 c.c. filtrate showed a concentration of hydrochloric acid equal to $\frac{N}{25}$.

10th November—30 c.c. filtrate showed a concentration of hydrochloric acid equal to $\frac{N}{47}$.

15th November—To find out whether the pancreatic duct was still occluded the abdomen was again opened over the pylorus, an inch to the right of the previous incision. On opening the peritoneum and drawing out the pancreas and duodenum, the duct was found tied with the silk ligatures which had been formerly used. There was a small adhesion of peritoneum and omentum just over the duct. The duct was now ligatured in two places with silk, and cut between the ligatures, when it was seen that it had become patent, showing that simple ligature is not sufficient to interfere with function, as has been noted in ligature of the Fallopian tubes.

18th November—Recovery was complete. 30 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{23{\cdot}7}$.

20th November—18 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{75}$.

21st November—24 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{21{\cdot}4}$.

28th November—The abdomen was again opened in the mid-line on the site of the first incision. Some adhesions were found in the peritoneum, but not of any moment. The peritoneum, however, was rather thin. The dog was well nourished and in excellent condition.

The common bile duct was ligatured in two places with silk at its entrance into the duodenum, and divided between the ties.

29th November—Animal lively, and with good appetite.

2nd December—40 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{20}$.

3rd December—14 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{18}$.

4th December—20 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{17{\cdot}8}$.

7th December—10 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{10{\cdot}8}$.

13th December—20 c.c. gastric juice showed a concentration of hydrochloric acid equal to $\frac{N}{17{\cdot}8}$.

A few days after tying the common bile duct jaundice began to manifest itself by the usual yellow discoloration, which was first evident on the abdomen, spreading thence over the thorax, inside the legs, and up into the neck, becoming deeper in colour as time went on.

As the dog was a white one the icterus showed itself with great plainness, but the general health of the dog appeared in no wise impaired—at least, for the period during which it was under observation—for the animal was as frisky and playful as before, and as eager for its meals.

The faeces were pasty in consistence, and of a slaty colour, and the urine gave the reactions of bile pigment and bile salts in a marked manner.

The dog was fed with meat on the 18th December at 1.30 p.m., and again at 2 p.m. on the 19th, and was killed at 4 p.m. on the 19th.

Post-mortem Examination.—The gall bladder and ducts were enormously distended with dark green bile, which was becoming inspissated in the gall bladder. All the ducts within the liver were dilated and filled with fluid, dark green bile, and even sections of the liver gave a green oosing of bile.

The intestinal contents were exceptionally fluid, and the first 2 feet 8 inches of small intestine measured from the pylorus contained food of acid reaction.

The next 3 feet of gut was empty, but the reaction of the moist mucous membrane was acid.

The next 2 feet contained the meal of the 18th instant, and was alkaline.

Microscopical Sections of Liver and Pancreas.—With the possible exception of an

increased distinctiveness of the nucleoli when stained with Eosin, sections of the pancreas showed nothing abnormal.

Liver sections also had a normal aspect, the bile ducts having apparently assumed their normal dimensions through elastic retraction.

The next point to determine was whether prosecretin actually existed in the duodenum and jejunum of the animal. If it were absent, then a high degree of acidity could be maintained in the chyme entering the gut and yet the liver and pancreas remain unstimulated, supposing these organs were still responsive to secretin.

The duodenum and jejunum were, therefore, washed and the mucous membrane scraped off with a knife. The mucous scrapings were ground with sand in a mortar, digested with diluted hydrochloric acid, boiled, and whilst boiling made slightly alkaline with potash, and then just acid with acetic acid. On filtering a somewhat opalescent filtrate was obtained, which was now tested for secretin.

A dog was anaesthetised with chloroform, and ether, preceded by morphia injection. Blood pressure was recorded in the usual way from the carotid. The external jugular vein was cannulised for purposes of injection. A cannula filled with water and connected with a rubber tube with a glass nozzle (both filled with water) was next tied into the pancreatic duct.

No flow of pancreatic juice was obtained.

On injection of 5 c.c. of the mucous membrane extract a fall in arterial blood pressure was produced. As the blood pressure began to recover a marked flow of pancreatic juice was observable in the cannula, the drops rising to 10 per minute.

This experiment proves, then, that prosecretin was undoubtedly present in the mucous membrane of duodenum and jejunum, and if this was so, then the acid of the chyme must of necessity have produced secretin, which, entering the blood stream, would eventually reach both pancreas and liver.

We are at this point confronted with two important questions :— First, does secretin, when it enters a pancreas or liver with duct ligatured, excite the organ to further secretory activity ; and second,

is secretin removed from the circulation to a less extent by a pancreas or liver with ligatured duct than by the normal organ ?

If the first question be answered in the affirmative, then the greater degree of acidity in the stomach the greater will be the stimulus which the pancreas and liver receive. It must also be noted that the pancreatic juice (and to a less extent the bile) is prevented from reaching the duodenum, the acid of the chyme remains longer unneutralized, and will form secretin for a longer time.

It is difficult to believe that the pancreas or liver continues to be equally susceptible to secretin stimulation after ligature of the duct—in fact, the slow return of colour to the faeces when obstruction causing icterus is removed points pretty clearly to the fact that this response to stimulation has been considerably reduced, and is but slowly regained. Moreover, a recent investigation of this subject by Zunz and Mayer[1] has demonstrated the fact that twelve to fifteen days after ligature of its duct the pancreas could still be excited by secretin, but lost this power later.

If the obstructed organ does not remove secretin from the circulation to the same degree as the normal organ, then more secretin is available for any organ responsive to this hormone and one which is in a normal condition. In the case in point more secretin would be at hand for the stimulation of the intestinal glands even if the concentration of acid in the stomach remained unaltered.

Digestion in Jaundice

When pancreatic juice is kept back from entering the gut, and thus digestion has to proceed in the absence of trypsin, amylopsin, and steapsin, it has been found that a compensatory hyperactivity of the intestines is produced[2] which allows the animal to eat and assimilate ordinary food. This compensation must be still more marked when the bile is also prevented from mixing with the intestinal contents, for bile, by reason of its alkalinity, takes some share in

1. *Biochemisches Centralblatt*, Vol. IV, p. 577, 1905.
2. Zunz and Mayer, *Centralblatt f. Physiologie*, Vol. XVIII, 364.

neutralising the acid chyme; hence in its absence after pancreatic obstruction the whole brunt of neutralising the chyme falls on the succus entericus, and until neutralisation is effected secretin will continue to be formed, and the intestinal glands continue to be stimulated.

It is obvious, too, that in such cases peptic digestion will be much more prolonged in duration, for instead of ceasing in the duodenum it will be extended as far down the intestines as the chyme exists unneutralised.

Now, the enzymes present in the intestinal succus are :—

(*a*) Erepsin, which transforms the products of peptic digestion, namely, albumoses and peptones, into the same acid products as occurs with digestion with trypsin. Erepsin also acts on the unhydrolysed proteids caseinogen and fibrin.

(*b*) Lactase, Maltase, and Invertase, which act on the three disaccharides taken in food and break them down into the monosoccharide sugars, dextrose, levulose and galactose.

Ferments acting on starch or dextrin, fats, and simple proteids, such as albumen and globulin, appear to be unrepresented in the succus entericus, and it is highly improbable that they should be formed *de novo* in the compensatory activity mentioned.

The suggestion has been made that during pancreatic obstruction the ferments formed by that organ are absorbed by the blood and re-excreted in the intestinal juice, but no experimental confirmation of this statement has been advanced. Even if steapsin were thus excreted in the succus the absence of bile salts would prevent absorption of all fatty acids except those like butyric acid, which have a measurable solubility in water. It is much more probable that the digestion of fat which has been detected after ligature of the pancreatic duct was due to bacterial saponification, but in such cases bile was present to dissolve fatty acids of high melting point and low solubility. If bile is absent then, no matter how efficient bacterial hydrolysis may be, only the fats compounded of acids soluble in water can be assimilated.

Whether a diastatic enzyme is present normally in the succus

entericus is a somewhat dubious point; if it be absent, then if the pancreatic secretion be withheld all starch or dextrin which has escaped digestion in the mouth would remain unabsorbed in the bowel. If, however, it does appear normally or can appear in special circumstances, the compensatory activity of the intestines would surely include this important element in digestion.

One may state, therefore, that when the pancreatic and bile ducts are obstructed normal digestion will still continue to be carried out by the intestine with respect to sugars, proteids which have been changed into albumoses and peptones by the gastric juice, also fibrin and caseinogen even if unaltered by the stomach. The presence of bacteria will confer some slight powers of fat digestion and possibly of starch digestion.

In the light of these facts the increased acidity of the stomach contents noted in the dog operated on would have as consequence—

(1) An increased excitation of the glands of the small intestine brought about by secretin.

(2) An increase in the digestive powers of the stomach by which more proteids can be transformed into products upon which the succus entericus can act.

(3) A possible increase in the extent of the tract in which peptic digestion takes place.[1]

As has been stated, the pancreas and possibly the liver become at length insensitive to secretin stimulation, so that a rise of gastric acidity and a consequent rise in the production of secretin would not injuriously affect them.

Summary

1. In the animal operated on (dog) ligature of the pancreatic duct was in one case followed by a temporary cessation of hydrochloric acid secretion in the stomach; in a second operation this was not observed.

1. It is interesting to note that the Russian investigator, Schegalow (Maly's *Jahresbericht*, Vol. XXXII, 396, 1902), found an increased secretion of gastric juice to be consequent upon ligature of the pancreatic duct.

2. Subsequent obstruction of the bile duct in the same animal was followed by an actual rise in the hydrochloric acid secretion of the stomach.

3. After such double ligation, prosecretin still remained present in the duodenal and jejunal mucous membrane.

4. The contents of the duodenum and jejunum were found to be acid for a distance of 5 feet 8 inches; beyond this point an alkaline reaction was obtained.

5. Merely ligaturing the pancreatic duct failed to produce a permanent obstruction, though the ligature remained tightly drawn and unabsorbed.

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